MISCONCEPTIONS, CONCEPTS AND APPROACHES IN RHIZOSPHERE BIOLOGY

G.D. Bowen

Division of Soils, C.S.I.R.O., Glen Osmond
Adelaide, Australia

INTRODUCTION

I believe the last few years have heralded a new era in the study of microbial growth around roots. We have entered a period of increasing concern with population dynamics of the rhizosphere and the interactions which occur there, an increasing concern with the mechanisms involved, and an increase in hypothesis formulation and experiment and in simulation modelling approaches. A large background knowledge of the rhizosphere has been built over many years by direct microscopy and by traditional isolation methods, but these studies tended to be static, not dynamic. As Newman (1978) pointed out, there are a number of questions related to the rhizosphere which conventional studies have not answered. These include questions such as: "What are the specific growth rates of microorganisms in the rhizosphere?", "How do growth rates change with the age of the root?" and "What are the sources of inoculum for roots; what are the quantitative relations between populations of mycorrhizal fungi in soil, infection of the root and plant response?".

The interest in rhizosphere biology originates mainly from effects of soil organisms on plant growth. For example, if we are to realize fully the potential of symbiotic microorganisms to stimulate plant growth we need to ensure that a selected, highly efficient symbiont will establish and persist in the face of competition from possibly less efficient naturally occurring symbionts (Bowen, 1978) and of antagonism from other soil organisms - "biological control" of symbionts is just as real as biological control of plant pathogens. We also need to know appropriate strategies to increase the populations of highly beneficial organisms where they occur naturally, for example, symbionts and organisms suppressive to root diseases. This involves an appreciation of factors which selectively enhance populations of particular microorganisms. We need to learn appropriate management techniques to decrease the impact of root diseases and this requires a knowledge of their epidemiology. The possibility of rhizosphere microbial capture of plant nutrients, of nitrogen fixation by free living organisms and of possible detoxification of allelopathic substances (Newman, 1978), makes an understanding of energy sources available to microorganisms necessary, and how these are affected by soil and plant factors.

Perhaps the most important force in the "renaissance" of rhizosphere ecology is the application of skills derived from other fields. Thus the use of scanning electron microscopy (Campbell and Rovira, 1973; Elliott, Gilmour, Cochran, Coby and Bennett, 1979) and especially transmission electron microscopy (TEM) of the root-soil interface, coupled with histochemical techniques, (Foster and Rovira, 1978; Rovira, Bowen and Foster, 1980) have allowed a more direct examination of the spatial and physiological relations between roots and microorganisms associated with them. These studies have also indicated the possibility of unique types of microorganisms in the rhizoplane (Foster and Rovira, 1978). The use of isotope labelling techniques with plants growing in soil (Barber and Martin, 1976) have allowed a better appreciation of the amounts of substrates coming from roots under almost natural conditions. Conceptual models of complex situations, such as the epidemiology of rhizosphere colonization and the identification of parts which are amenable to simple experimentation in soil (Bowen, 1979; Bowen and Theodorou, 1973), have allowed an evaluation of factors affecting the various components. The application of population dynamics, and of ecological and modelling approaches (Newman and Watson, 1977; Bowen and Rovira, 1976), well developed in other fields, have made rhizosphere biology a more quantitative science.

In this paper I wish to examine some of the more recent advances in rhizosphere biology and how they have moulded our present concepts. The broad conceptual model I shall use is that of Figure 1, which is discussed in detail elsewhere (Bowen, 1979). This is an ecological type model at the plant-soil level and is not appropriate to consideration of physiological mechanisms or of the details of microbial interactions. Figure 1 indicates:

i) that the plant is the main driving force for the system and thus factors affecting its growth will also affect microbial growth;

ii) that distribution of roots and microbial movement to them are essential considerations in understanding the composition of the root microflora;

iii) that microbial growth at the root surface can itself affect losses from the root and that microbial growth at the surface may affect substrates available for organisms away from the root surface.

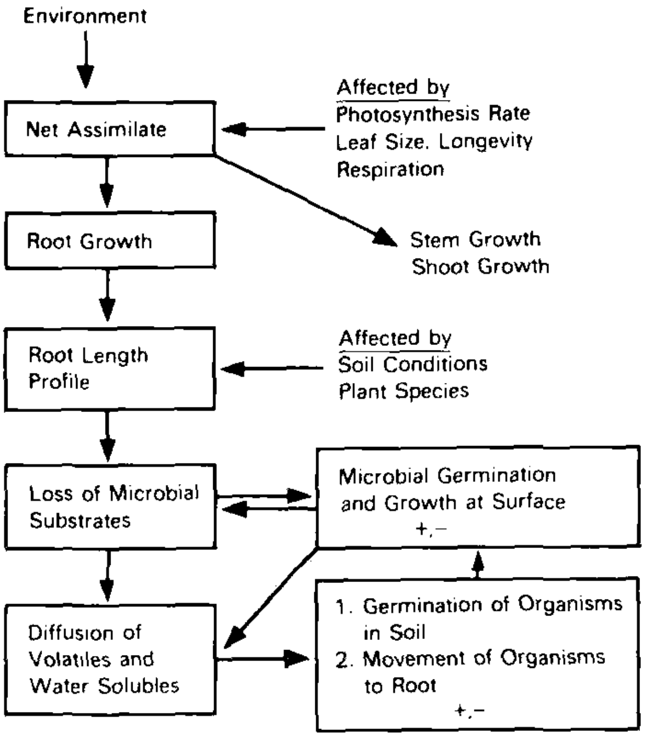

Fig. 1. A simple conceptual model of plant microorganism interactions.

SOME MISCONCEPTIONS

Old ideas do not die readily and the years have led to a number of firmly entrenched misconceptions on plant-microbe interactions and rhizosphere biology.

The main misconception is that studies of microbial growth, physiology and interactions in laboratory media or with plants in culture solutions are highly meaningful for plants growing in soil. Some of these studies may possibly be relevant, for example, the production of highly specific chemicals (Krupa and Fries, 1971), but usually they are not. This erroneous belief ignores the quite different physical and chemical nature of soil and of substrates coming from roots growing in soil compared with solution cultures (which are often luxurious in nutrients). Theodorou and Bowen (1971) found that some strains of ectomycorrhizal fungi which grew well on laboratory media at 16°C could not colonize the rhizoplane of *Pinus radiata* at this temperature in sterile soil, although they did at 20°C and 25°C. Also, antibiosis in laboratory media often has little or no relevance to antibiosis in the rhizoplane, either for plant pathogens (Broadbent, Baker and Waterworth, 1971) or ectomycorrhizal fungi (Bowen and Theodorou, 1979) except possibly for organisms showing consistently strong effects (Marx, 1969). Antibiosis in laboratory media is influenced markedly by different media. Therefore there is no real alternative to studying microbial growth dynamics and interactions in soil itself. This is not to deny that studies in solution culture are almost essential (initially) in investigations at a cellular physiology level, for example, recognition between roots and symbionts, and the control of transfer of metabolites between microorganisms and roots. The use of sterile soil in some experimental approaches to epidemiology and microbial ecology of the root itself introduces artifacts. However this is closer to reality than solution culture systems and enables one to isolate effects of soil physical and chemical variables from those of other soil microorganisms.

A second entrenched misconception is that the dynamics of microbial growth and interactions in well stirred laboratory solutions apply to microorganisms in soil and on the root. As will be seen below, this is not so. This assumption ignores that these environments are usually comparatively low in substrates, that solute transfer limitations occur in such discontinuous matrices as soil, and that growth dynamics on surfaces are probably quite different from those in solutions. Prey-predator relations also will be affected by the spatial restrictions of surfaces and soil.

A third common misconception, particularly relevant to symbiotic relations of plants, is that natural selection will have led to the dominance of the most effective strains of rhizobia or mycorrhizal fungi in an area. However, the trend of evolution has been for survival, not high productivity, and it is more likely that indigenous strains of these symbionts have evolved primarily to be compatible with the existing soil physical chemical and biological environments. There are several instances where the naturally occurring symbionts are not the most effective (Mosse, 1975).

GROWTH SUBSTRATES

Over the last few years concepts of substrates released from roots for microbial growth, previously based on leakage of exudates from solution-grown plants under sterile conditions have changed in three major respects:

i) It is now recognised that substrates from roots have

many origins. Rovira, Foster and Martin (1979) have classified these as:
exudates of low molecular weight leaking from intact cells;
secretions released actively from cells;
mucilages of various types;
mucigel consisting of microbial and of plant mucilages (possibly modified by microbial action) and;
lysates from autolysis of senescing cells.
Sloughed root cells should be added to these (Griffin, Hale and Shay, 1976).
By using techniques specific for 1,2-diglycol groups in sugars linked between carbon 1 and 4, Foster has distinguished between gels produced by roots and gels produced by microorganisms. Furthermore, differential hydrolysis has indicated fungal gel is more stable than root derived mucilage (see Rovira, Bowen and Foster, 1980). This may be a reason for the increase in aggregation of soil caused by vesicular-arbuscular mycorrhizas (v.a.m.) (Sutton and Sheppard, 1976).

ii) In sharp contrast to losses of substrate from solution grown plants, losses from soil grown plants can be very large indeed. Using ^{14}C- carbon dioxide labelling, Barber and Martin (1976) showed losses into soil of 5-10% of the assimilated carbon by three-week wheat plants. In four experiments on wheat and barley by Barber and Martin (1976) and Martin (1977a), between 3 and 9% of the carbon in roots was lost as water soluble substances and 17-25% was water insoluble material. Newman (1978) concluded soluble substrates from roots are frequently 1-10% of the root weight and may sometimes reach 25%.

iii) The "normal" soil microflora increases losses from roots by up to 100% (Barber and Martin, 1976). Martin (1977a) found a loss of 22-24% of the assimilate over the first 6 weeks from wheat plants grown in non-sterile soil via the roots. The carbohydrate budget of mycorrhizal plants is receiving increasing study (Harley, 1975; Bowen, 1978) and it is now apparent that losses of assimilates from roots due to the non-infecting soil microflora must be considered as an important factor in carbon balances of soil-grown plants. This increased loss of assimilates due to microorganisms raises at least two important questions:

(a) Is the rhizosphere microflora increase of assimilate loss a significant enough drain on the plant to reduce its growth or does the plant compensate by photosynthesizing more? A similar question has been asked with respect to symbionts (Bowen, 1978) Increases in plant growth following soil sterilization which are not explicable readily by control of pests and diseases or by nutrient release, could be due to the effect of the rhizosphere microflora draining away organic compounds which otherwise might be utilized by the plants. In symbiotic systems the benefits normally far outweigh the assimilate costs but even here, growth depressions may occur either before the plant benefits from the symbiosis (Cooper, 1975; Gibson, 1966) or where the symbionts are superfluous (Crush, 1976). It has been calculated that anything from a few percent to as much as a quarter of a tree's assimilate may be used by ectomycorrhizas (Newman, 1978).

(b) What are the origins of the increased assimilate loss in the presence of microoganisms? Martin (1978) considered the increase was due to microbial lysis of cells. Light microscopy and TEM studies show heavily colonized individual senescent cells near the root apex (Bowen and Foster, 1979). However, we do not know what is cause and effect in this instance, for no studies have been made on the frequency of senescent cells with sterile and with non-sterile roots. Even if sub-clinical pathogens, causing cell senescence prematurely, are the main reasons for the increased substrate losses, there are at least two other ways in which substrates could be lost locally to the advantage of microorganisms there.

i) Microbial absorption of exudates could maintain a high concentration gradient at that point and sustain diffusion out of the cell in a parallel way to the sustained diffusion of glucose and fructose from higher plants to mycorrhizal fungi (Harley, 1975).

ii) Local production by some microorganisms of compounds increasing cell permeability would increase rates of exudation at that point. This would give a particular competitive advantage to the organisms concerned and would be an especially useful property of late arrivals at the root surface. It would also be useful in colonization of cell surfaces (away from cell junctions) where exudation appears to be low. This hypothesis could be tested by electrophysiological approaches or by examining efflux from roots radioactively labelled with ions such as chloride which are lost passively. A number of organisms associated with roots are able to produce hormone type compounds, for example, some ectomycorrhizal fungi (Slankis, 1973). Highinbotham (1968) found hormones increased permeability of *Avena* coleoptiles, but several other compounds may also have this effect. If the hypothesis above is confirmed, selection of organisms with such a property might aid in their establishment.

Factors affecting plant growth and composition (Fig. 1) and factors affecting membrane permeability, would be expected to affect substrates coming from the roots and a closer integration of the basic fields of plant physiology and microbiology is necessary. For example, Reid and Mexal (1977) found not only did roots of *Pinus contorta* exude 27-29% more ^{14}C- labelled assimilate at -400 KPa water potential than at -200 KPa, but also 45% more assimilate was translocated to the roots. Some roots exuded over 80% of the total ^{14}C-activity which reached them in 10 days. Similarly Martin (1977b) found wheat roots lost twice as much water-soluble material in water-stress conditions. Phosphorous deficiency increases losses of amino acids and reducing sugars from roots by 2-3 fold (Bowen, 1969; Ratnayake, Leonard and Menge, 1978) and two mechanisms may be responsible. Ratnayake *et al.*, (1978) found evidence for increased permeability of low phosphate roots of 8-10 week old seedlings or sorghum and citrus. Using essentially the same method on 2-4 week old *P. radiata* roots Bowen (1969) found no evidence for increased permeability but found a doubling of free amino acids and amides in phosphate deficient roots. Presumably the large phosphorus supplies of *P. radiata* seed were sufficient to maintain complete root integrity for at least 4 weeks. Barrow (1977) found no growth response of this species to applied phosphate until 9 weeks after application of phosphate. It would be interesting to compare total exudates from closely related C_3 and C_4 plants.

The other basic determinant of substrate distribution at the root soil interface is the diffusion of substrates through soil. Newman and Watson (1977) considered this in a model in which microbial abundance in the rhizosphere was simulated. The growth rate of microorganisms at each point in soil was assumed to be controlled by the concentration of soluble organic substrate. The concentration of substrate changed due to:

i) its production by the root and diffusion through soil,

ii) its production in the soil by breakdown of insoluble organic matter and

iii) its use by microorganisms.

In the Newman-Watson model diffusion of substrates through wet soil was rapid enough for extensive microbial development as far out as 4 mm. In drier soil (-0.3 bar) some exudate initially diffused into soil thus stimulating microbial growth but as populations developed at the root surface they captured more of the exudate and much less diffused into soil. At -0.3 bar soil matric potential, microbial abundance at the root surface was 15 times that at 0.1 mm and 430 times that at 0.8 mm at 10 days. With lower diffusion coefficients of substrates in soil, that is, at -1 bar matric potential, still a moisture conducive to good root growth, the gradients of microbial abundance with distance from the root were even more steep. If this model can be validated, clearly it has great practical use in predicting the distances from the roots at which propagules may receive enough substrate to germinate under various conditions. The limited diffusion of soluble substrate predicted by the model suggests that volatiles may be very important in stimulating microbial activity more than 1 or 2 mm from the roots. In Newman and Watson's model a 10 fold difference in exudation led to a 120 fold difference in microbial abundance.

This model, like any model, needs experimental testing and possibly later refinement in critical areas. The data of Bowen and Rovira (1973) are remarkably consistent with it. The steep fall in bacterial numbers with distance from the root is also confirmed by the TEM data of Foster and Rovira (1978) in which, at 15-20 μm from the root, the frequency of bacteria declined to 10% of that at the rhizoplane. TEM techniques should be invaluable in testing predictions of such models.

It should be remembered that predictions by models are not proof and they, therefore, need validation. One of their powers is they indicate the most sensitive components of the system for further research and testing and the areas possibly most responsive to manipulation. Some aspects of modelling in rhizosphere ecology are discussed by Bowen (1979).

A major limitation of root substrate studies so far is that they have usually measured gross effects and have not dealt with heterogeneity between and along roots. Approaches such as split root techniques and potometer techniques (often used in ion absorption studies) could be useful in this respect with soil grown plants. Microbial assays have demonstrated the types of differences which can occur between and along roots: differences in microbial colonization of seminal and nodal roots of wheat have been shown by traditional plating methods (Sivasithamparam, Parker and Edwards, 1979); that the junctions of root cells with each other are the major sites of exudation has been shown by inoculating roots uniformly with organisms and subsequently observing sites of colony development (Bowen, 1979); that older parts of roots may provide as much substrate as younger parts has been shown by studying microbial growth rates on different parts of washed, sterile roots planted to non-sterile soil so that all parts were exposed to microorganisms at the same time (Bowen and Rovira, 1973).

RHIZOSPHERE POPULATION DYNAMICS

As Figure 1 shows, the microbial population of the rhizosphere will be determined by factors such as rooting intensity, the germination of propagules in the soil and their growth to the root, and growth on and along the root. One of the major deficiencies of rhizosphere biology is the very small numbers of studies *in soil* on any of these microbial parameters. Studies on spore germination provide several good examples of the importance of performing such studies in soil: Daniels and Duff (1978) found no germination of spores of three isolates of v.a.m. fungus on agar but 35-45% germination in soil. Most studies of germination of basidiospores in laboratory media have shown very low rates of germination, for example, often less than 1.0% (Fries, 1966), but Theodorou and Bowen (1973) obtained approximately 10% germination of basidiospores of an ectomycorrhizal fungus added to planted soil. There appear to be no experiments yet on germination of spores of ectomycorrhizal fungi on plant roots in soil. Simple methods are available (or can be devised) to study the above factors in soil (Bowen, 1979) without resort to laboratory media or use of buried slides (or cellophane) which bring their own artifacts (Wong and Griffin, 1976).

The short generation times of organisms in rich, stirred laboratory media have not been observed on roots. In what is one of the only existing studies on growth rates of bacteria on roots in soil, Bowen and Rovira (1976) found generation times of 5.2 h and 39 h on *P. radiata* roots in soil, for a *Pseudomonas* sp. and a *Bacillus* sp. respectively, compared with 77 h and greater than 100 h in nearby soil. Barber and Lynch (1977) recorded a "doubling time" of 24 h for total bacterial counts with barley in solution culture. Such generation times are by no means uncommon in natural systems. Generation times should be used in rhizosphere biology rather than the usual root/soil (R/S) ratios because :

i) this is a more dynamic parameter than the static measurements usually made with rhizosphere studies,

ii) this brings rhizosphere biology into line with other fields of population biology and

iii) because the generation time concept is internally consistent and a more valid measurement than comparing growth on a surface (root) with that in a volume (soil).

The relatively rapid growth of pseudomonads on the root in the studies by Bowen and Rovira (1976) could explain why they are a major group of organisms in the rhizosphere. However, there are very large growth differences between closely related strains of pseudomonads. For example, the relative increase in four strains of *Pseudomonas* sp. inoculated to *Eucalyptus sieberi* were 2, 62, 141 and 380 over a period of four days. On *E. globulus* the increases were approximately one sixth of those above (Bowen, 1979 and unpublished). What are the bases of such large differences in growth between closely related bacterial strains? Are they due to different abilities to use common growth substrates or do they reflect the needs of some strains for particular growth factors not supplied by a plant species? Such a reason seems more likely, at least in the case of the ectomycorrhizal fungi which showed marked differences between strains (of the one species) in the rhizosphere at 16°C, although all grew similarly in laboratory media at this temperature (Theodorou and Bowen, 1971). The very large differences in growth in the rhizoplane between mycorrhizal fungi (Bowen and Theodorou, 1973, 1979) and between rhizobia sp. (Chatel and Greenwood, 1973) gives appreciable scope for selection of strains which can colonize particular species of plants rapidly.

Contrary to earlier ideas that the rhizoplane is entirely covered by microorganisms, only 4-10% of the surface is usually occupied (Bowen and Theodorou, 1973, Rovira Newman, Bowen and Campbell, 1974), except for such associations as ectomycorrhizas in which short lateral roots may be completely sheathed by fungus. Ecological sampling methods developed in other fields and used by Newman and H.J. Bowen (1974) and by Malajczuk and G.D. Bowen (see Bowen and Rovira, 1976) have shown a non-random distribution of organisms on the root and a preferential colonization of the junctions of cells. There are 50 times as many bacteria in this position with *E. calophylla* roots as in other areas (Bowen and Rovira, 1976). The cell junctions are the major sities of exudation and the major routes of spread of initial colonization but even so, colonization of them is not complete.

The incompetences of root cover by microorganisms is a reflection of the often limited migration of organisms along the root and the relatively wide spacing in soil of inoculum for new root growth. A root will frequently grow faster than existing colonies can spread (Bowen and Rovira, 1973) and new root growth may be colonized largely from the soil. How far do propagules move to the root? This is probably related to size of propagule and the reserves they contain (Bowen and Rovira, 1976).

Microbial propagules differ considerably in size from bacteria, 1-4 µm in size, to small fungal propagules of only a few microns diameter to larger spores, such as the spores of v.a. mycorrhizal fungi are commonly 200-400 µm diameter and higher, to propagules such as sclerotia, which are 1-2 mm and larger. Two main reproduction alternatives appear to be followed in fungi, namely the production of many small propagules or of fewer large propagules. This appears reminiscent of *r* and *K* strategies respectively in other fields of ecology (see Southwood, 1976). In short, *r* strategists are basically opportunistic, with rapid growth rates; as the habitats they colonize are often virtual ecological vacuums, high competitive ability may not be required and they are typically small in size; mortality rates may be high but they recover quickly from population reduction. *K* strategists, by contrast, have large size and, longevity, low mortality rates, high competitive ability and a large investment in each offspring and may recover slowly from reductions in population. (This analogy may be a rewarding one also in considering the biological control of diseases - biological control would be expected to be more feasible on *K* organisms or the *K* stage of organisms which can go from *K* to *r*, for example, zoosporangia of *Phytophthora* producing zoospores).

Bacterial migration to the root in a film of water is quite limited below field water capacity (Bowen and Rovira, 1976). Therefore initial colonization of a new root surface in the absence of free water will usually be by the chance touching of some of the many small (*r* type) propagules, namely, bacteria and small spored fungi with rapid germination and growth on simple substrates. Thus while one can predict the main types of organisms which will be the primary colonizers of the rhizoplane, the actual species composition will be largely a probability exercise. Colonization by organisms further from the root may take longer. *K* strategy organisms with fewer, larger propagules capable of growth over further distances to the root are particularly suited to grow longer distances and may be the major component of the "late" colonizers, especially with plant species with low rooting intensity. *K* strategy organisms in other fields of ecology often have a high competitive ability and this might be expected also with the *K* type microorganisms suggested here.

Initial colonization of roots is often associated with organic matter and as pseudomonads are often in the organic matter their frequent occurrence in the rhizosphere is not surprising. The importance of organic matter as a source of inoculum suggests a close link between organisms colonizing one season's roots and the next. The judicious selection of previous crops therefore may be one avenue of increasing the inoculum potential of symbionts (for example, rhizobia with non-legumes, Diatloff, 1969) or of root disease suppressing organisms. Thus grass roots stimulate high populations of *Phialophora radicola* var. *graminis* which is antagonistic to *Gauemannomyces graminis* var. *tritici* (Slope, Sah, Broom and Gutteridge, 1978). Also changes of the microbial composition of soil are likely to be rapid with crop sequences with large root biomasses in the first few years, for example, with grasses rather than with trees (Bowen, 1979).

Although there is some evidence (Dazzo, Napoli and Hubbell, 1976) that adsorption of rhizobia to roots may be related to host specificity in *Rhizobium*-clover symbioses, it is not necessary to invoke lectins (or other recognition phenomena related to *infection*) in growth specificity around roots.

Many of us are used to thinking about monocultures of plants, yet most natural systems and many agricultural pasture systems are mixed. It is important, therefore, that we recognise that biological interactions at the plant-plant-microorganism level also occur. Thus Iqbal and Qureshi (1971) reported that the crucifer *Brassica campestris* reduced v.a.m. infections of field grown wheat and reduced wheat yield by 25%. Christie, Newman and Campbell (1978) found *Lolium perenne* to decrease v.a.m. infection of *Plantago lanceolata* by almost 40% and a pasture mixture increased bacterial and fungal growth on *Plantago* by over 200%. Such important effects warrant further study especially with interactions between plants and "economic" microorganisms. As Christie, Newman and Campbell (1978) pointed out, some of the effects may have been indirect, arising from changes in plant nutrient status, assimilation and root exudation of a plant when grown with other plants. However not all effects were explicable in this way and direct effects might occur, for example, a substance from *Calluna vulgaris* can inhibit ectomycorrhizal fungi of *Picea abies* in heathland (Robinson, 1972).

Models for plant response

The epidemiological considerations above are of particular importance in predicting the impact of known populations of diseases, (that is, predicting the necessity of employing expensive control measures) and in predicting situations in which appreciable responses would be obtained by inoculation with mycorrhizal fungi (possibly with some added fertilizer) even when naturally occurring fungi are of an efficient type. Empirical or mechanistic models could be developed for these situations. I have made the simplifying

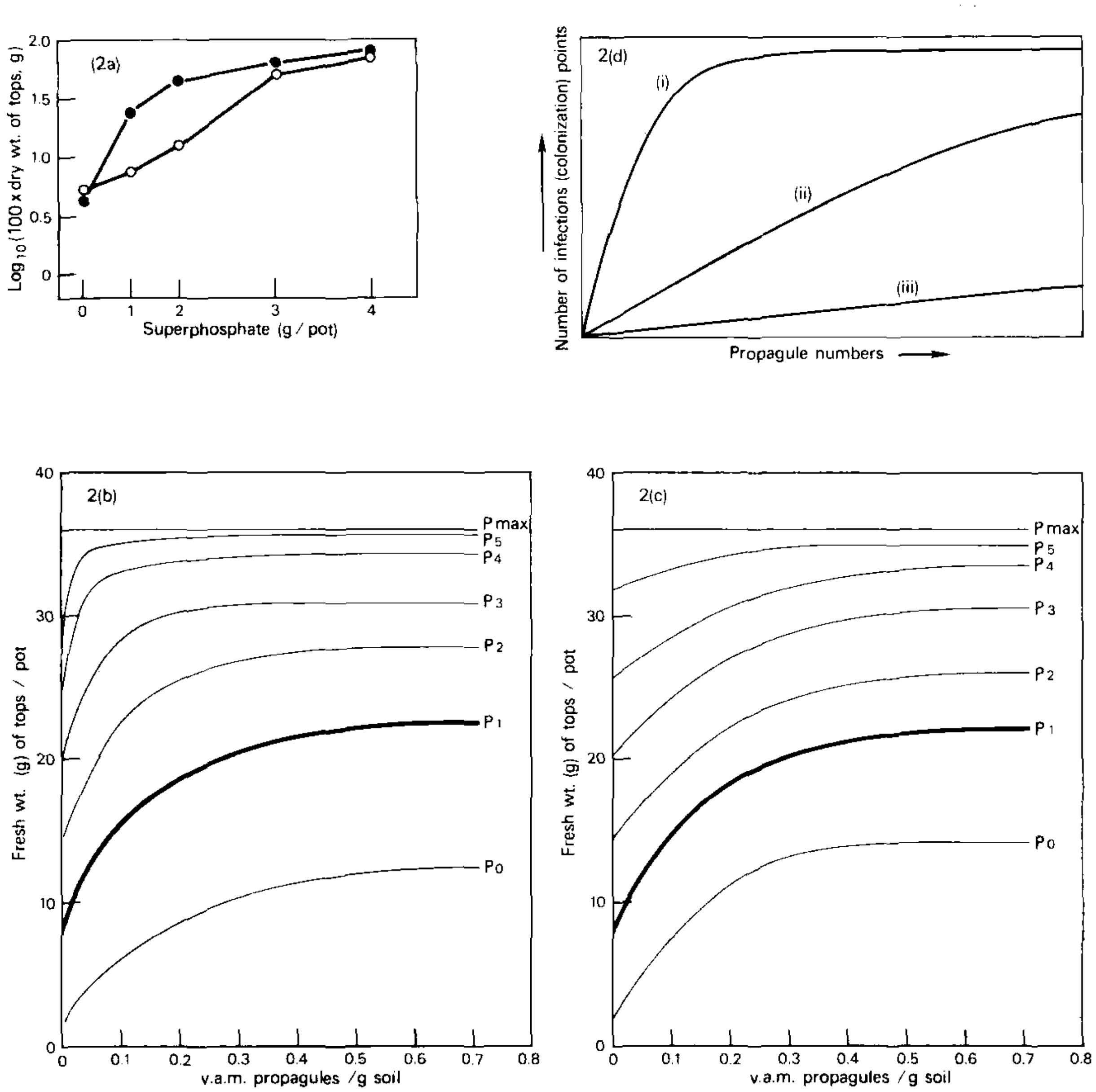

Fig. 2. (a): Response of subterranean clover to v.a. mycorrhiza infection at 5 soil phosphate levels; mycorrhizal (●), non-mycorrhizal (○). (b) and (c): Possible response curves to varying mycorrhizal infection with different phosphate levels; P_1 is an experimentally derived curve on Medicago truncatula (Bowen and Smith, unpublished observations) (d): hypothesized number of infection or colonization points with varying populations of propagules which move (i) over long distances to the root, (ii) moderate distances and (iii) small distances.

assumption that root diseases and mycorrhiza are "seen" by the plant as decreases or increases respectively in effective "root" length for absorption of nutrients (assuming no water deficits). I shall refer particularly to the mycorrhizal response.

An empirical model could be based on the type of response to mycorrhizal infection as given in Fig. 2a, from Abbott and Robson (1977) on *Trifolium subterraneum* with 50-100% of the root mycorrhizal. As the limiting nutrient, phosphate, increased, the infection became redundant. The question then becomes one of the infection needed to obtain the full mycorrhizal response at any level of added phosphate. The two possibilities are indicated in Fig. 2b and 2c, in which the dark line was experimentally obtained on *Medicago truncatula* at one level of phosphate. The infection-response curve and the number of propagules of the v.a. mycorrhizal fungus in soil were obtained by diluting soil with the same soil which had been sterilized, and later counting the numbers of infections (Smith and Bowen, 1979, and unpublished observations). The upper line (P_{max}) is the level of phosphate at which no response would be obtained from inoculation.

A more general mechanistic model demands a model of plant nutrition which generates rooting intensity. At present such a model would be generated by a mixture of mechanistic and empirical sub-models. The infection levels, as a function of numbers of propagules and the distance they move to roots, is suggested in Fig. 2d. The generation of plant response from this infection then depends on the extent of growth of mycelial into soil infection then extending the rooting system; considering the poor state of this subject, it would be necessary to use empirical values (for example, from Sanders, Tinker, Black and Palmerley, 1977). The more widely spaced the root the more dependent the plant is likely to be on mycorrhizal infection (Baylis, 1975).

MICROBIAL INTERACTIONS

Microbial interactions such as antagonism, competition and synergism in the soil and the rhizoplane are probably the most important, yet the least understood, phenomena in rhizosphere biology. These can determine the success or failure of introduced inoculants and the biological "control" of diseases. As the rhizoplane is occupied incompletely by microorganisms we have a number of spatially discrete small communities rather than one large interacting community. Despite this incomplete cover of the root, interactions between organisms do occur because they tend to occupy the same favoured sites, that is, the longtitudinal junctions of cells, and a general microflora has been shown experimentally to decrease the rate of spread of an ectomycorrhizal fungus along the root by approximately half (Bowen and Theodorou, 1973). That is, microbial communities in soil and on the root are best considered as being in many micro-islands and they tend to interact only when their "territory" is crossed, for example, lysis of germ tubes of fungi as they grow through soil. The many different types of organisms which occur on roots cannot all be considered to occupy different "physiological niches" as is sometimes thought; many may be occupying the same niche but at different parts of the root.

The types of interaction which can occur on the root are indicated in Table 1, derived from inoculation studies on plants in sterilized soils (Bowen and Theodorou, 1979):

i) Very large differences occurred between the mycorrhizal fungi (as they do between strains of the one fungus species) in colonization of the root. These differences may be important ecologically, for example, the slow growth of *Pisolithus tinctorius* (only one strain was studied) may be the reason why it appears to be the dominant ectomycorrhizal fungus only in extreme situations, such as mine wastes.

ii) Large depressions of growth can be caused by antagonism, for example, *P. fluorescens* in Table 1. Although all fungi were depressed, some, for example, *T. terrestris*, were not as severely affected as others. However, antagonisms in simple mixtures may not be expressed in more complex situations for in other studies (Bowen and Theodorou, 1979) a *Bacillus* sp. with no effect on colonization by *R. luteolus* overcame the marked inhibition by *P. fluorescens* toward this fungus even though it did not reduce the numbers of *P. fluorescens*.

iii) Reductions in growth may be caused also by competition for substrates in short supply. Other organisms are neutral and some, for example, *Bacillus* sp.2 may be stimulative. TEM studies (for example, Foster and Rovira, 1978) show communities consisting of microcolonies of different organisms. Are these necessarily competitive? Could they sometimes be mutualistic? Although competition is the conceptual backbone of much ecological thought it is extremely difficult to study under natural conditions. However there are experimental approaches to this which have developed in other fields. For example, replacement series and diallel analyses (Williamson, 1972) and microbiologists could use such approaches with profit.

The possibility of synergistic associations opens up interesting avenues for further study, not only with regard to the mechanisms of synergism but also with regard to applied aspects such as establishment of symbionts and their

TABLE 1

Rhizoplane Interactions between Mycorrhizal Fungi and Bacteria (Bowen and Theodorou, 1979)

	Length of Root colonized by fungi (mm)				
	Bacteria present				
	Exp. 1			Exp. 2	
	0	*Ps. fluorescens*	*Bacillus sp.* #1	0	*Bacillus sp.* #2
Rhizopogon luteolus	27.6	8.3	23.7	30.7	34.1
Suillus luteus	16.2	3.2	8.7	19.1	26.2
Thelephthora terrestris	22.4	12.9	18.1	12.1	11.2
Corticium bicolor	17.2	3.2	15.3	15.8	27.1
Pisolithus tinctorius	6.9	5.7	4.2	6.9	1.9
L.S.D. $P<0.05$		5.4			6.2
$P<0.01$		7.1			8.2

Experiments were conducted in sterile soil for 4 weeks at 20°C day (12 hr), 16°C night.

effects on plant growth. Azcon, Barea and Hayman (1976) reported an instance in which lavender plants inoculated with v.a.m. fungi and phosphate-dissolving bacteria absorbed more phosphate from soil fertilized with rock phosphate than those inoculated with v.a.m. fungi or bacteria alone.

Soil microfauna

The possible role of soil microfauna is an important omission from most studies on microorganism-plant interactions. However the impact of soil microfauna may be large: nematodes may enhance root disease severity (Meagher, Brown and Rovira, 1978), pests may attack legume nodules - a situation in which remaining nodules may sometimes compensate by being more active - and v.a.m. fungi and nematodes can be mutually suppressive (see Bowen, 1978).

Protozoans may play a particularly important part in controlling microbial activities in the rhizosphere, and in adjacent soil. Coleman, Anderson, Cole, Elliott, Woods and Campion (1978) have adopted an experimental approach to studying changes of bacteria, carbon and phosphorus in soil microcosms inoculated with bacteria, nematodes and amoebae and a related more rapid recycling of phosphorus in bacterial biomass. However amoebae do not decrease bacteria populations in soil to very low numbers and this is partly because of the ability of the bacteria to reproduce and replace cells consumed by predation (Habte and Alexander, 1978). The roles of protozoans in the rhizosphere await more study and the prey-predator relations may be quite different in the two dimensional matrix of the root surface from the three dimensional framework of soil. There are, indeed, suggestions from solution culture studies that amoebae may directly affect substrate availability from roots. Darbyshire and Greaves (1970) found addition of an amoeba led to a large increase in the population of a *Pseudomonas* sp. on the roots of the grass *Phleum pratense* in solution culture although the reasons for this were not studied.

CONCLUSION

There is a need, not merely to understand systems and their controlling factors ("descriptive" science) but also to manage them if possible ("manipulative" science). Microbial ecologists are now beginning to come to grips with the task of understanding microbial growth around roots, but this is just a beginning and there is much more to be done on each of the aspects I have referred to above. Perhaps the major advance of the last 5 to 10 years has not been the application of new skills, important though this has been, but the questioning concepts of microbial growth derived from solution cultures which have been learnt by scientists during their traditional training. Instead, they are now examining what really happens in soil and on plant roots, that is, with 2-3 dimensional discontinuous systems, growth on surfaces, and situations in which diffusion of substrates into (and out of) the system may control growth.

There seems little point in trying to control the entire microbial composition of the rhizosphere and, given the largely stochastic nature of infection of new root growth, such an aim would be almost unattainable. However, there is great point in controlling the population of particular microorganisms and especially to increase the populations of beneficial symbionts. We have two general variables which could be manipulated, namely, plants and microorganisms, and in selection programmes we should take advantage of the variation in many properties of *each* of these. More avenues of management also arise because of the impact of non-host plants on colonization and infection of the root by symbionts.

High soil populations, high growth rates, resistance to antibiosis and high competitive ability appear to be key characters in successful establishment of particular microorganisms on the root. Organisms which locally increase permeability of the root cell may have a further competitive advantage. Establishment would be further enhanced by a selective or specific food source for the organism and this could be obtained by breeding (or engineering) both plants and symbionts for production and use respectively of unusual substrates. In fact the ultimate in "competition" would be highly specific host-microorganism combinations, for example, shortfalls in legume nitrogen fixation due to competition to inoculated rhizobia from poorly effective, naturally occurring rhizobia could be eliminated by breeding legumes so specific in their *Rhizobium* requirements that they nodulate only with the inoculated strain.

Although very significant advances have been made in microbial ecology of the rhizosphere in recent years, we still have much to learn. The new dimensions this field offers in plant physiology, physical chemistry of soil, growth at surfaces and population dynamics are challenging, exciting and highly relevant to plant-microbe interactions and plant productivity.

REFERENCES

Abbott, L.K., and Robson, A.D. (1977). Growth stimulation of subterranean clover with vesicular arbuscular mycorrhizas, *Australian Journal of Agricultural Research* 28, 639-649.

Azcon, R., Barea, J.M. and Hayman, D.S. (1976). Utilization of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and phosphate-solubilizing bacteria, *Soil Biology and Biochemistry* 8, 135-138.

Barber, D.A. and Lynch, J.M. (1977). Microbial growth in the rhizosphere, *Soil Biology and Biochemistry* 9, 305-308.

Barber, D.A. and Martin, J.K. (1976). The release of organic substances by cereal roots in soil, *New Phytologist* 76, 69-80.

Barrow, N.J. (1977). Phosphorus uptake and utilization by tree seedlings, *Australian Journal of Botany* 25, 571-584.

Bayliss, G.T.S. (1975). The Magnolid mycorrhiza and mycotrophy in root systems derived from it. *In* "Endomycorrhizas" (Ed. F.E.T. Sanders, B. Mosse and P.B.H. Tinker) pp.

Bowen, G.D. (1969). Nutrient status effects on loss of amides and amino acids from pine roots, *Plant and Soil* 30, 139-142.

Bowen, G.D. (1978). Dysfunction and shortfalls in symbiotic responses. *In* "Plant Disease" (Ed. J.G. Horsfall and E.B. Cowling), Vol. 3, pp. 231-256. Academic Press, New York.

Bowen, G.D. and Foster, R.C. (1979). Dynamics of microbial colonization of plant roots. *In* "Symposium on Soil Microbiology and Plant Nutrition" (Ed. W.J. Broughton and C.K. John) pp. 14-31. University of Malaya, Kuala Lumpur.

Bowen, G.D. and Rovira, A.D. (1973). Are modelling approaches useful in rhizosphere microbiology? *Bulletin of Ecological Research Communications*, Stockholm 17, 443-450.

Bowen, G.D. and Rovira, A.D. (1976). Microbial colonization of plant roots, *Annual Review of Phytopathology* 14, 121-144.

Bowen, G.D. and Theodorou, C. (1973). Growth of ectomycorrhizal fungi around seeds and roots. *In* "Ectomycorrhiza : Their Ecology and Physiology" (Ed. G.C. Marks and T.T. Kozlowski), pp. 107-150. Academic Press, New York.

Bowen, G.D. and Theodorou, C. (1979). Interactions between bacteria and ectomycorrhizal fungi, *Soil Biology and Biochemistry* 11, 119-126.

Broadbent, P., Baker, K.F. and Waterworth, Y. (1971). Bacteria and actinomycetes antagonistic to funal root pathogens in Australian soils, *Australian Journal of Biological Science* 24, 925-944.

Campbell, R. and Rovira, A.D. (1973). A study of the rhizosphere by scanning electron microscopy, *Soil Biology and Biochemistry* 5, 747-752.

Chatel, D.L. and Greenwood, R.M. (1973). Differences between strains of Rhizobium trifolii in ability to colonize soil and plant roots in the absence of their specific host plants, *Soil Biology and Biochemistry* 5, 809-813.

Christie, P., Newman, E.I. and Campbell, R. (1978). The influence of neighbouring grassland plants on each others endomycorrhizas and root-surface microorganisms, *Soil Biology and Biochemistry* 10, 521-527.

Coleman, D.C., Anderson, R.V., Cole, C.V., Elliott, E.T., Woods, L. and Campion, M.K. (1978). Trophic interactions in soils as they affect energy and nutrient dynamics. IV. Flows of metabolic and biomass carbon, *Microbial Ecology* 4, 373-380.

Crush, J.R. (1976). Endomycorrhizas and legume growth in some soils of The Mackenzie Basin, Canterbury, New Zealand, *New Zealand Journal of Agricultural Research* 19, 473-476.

Daniels, B.A. and Duff, D.M. (1978). Variation in germination and spore morphology among four isolates of Glomus mosseae, *Mycologia* 70, 1261-1267.

Darbyshire, J.F. and Greaves, M.P. (1970). An improved method for the study of the interrelationships of soil microorganisms and plant roots, *Soil Biology and Biochemistry* 2, 63-71.

Dazzo, F.B., Napoli, C.A. and Hubbell, D.H. (1976). Adsorption of bacteria to roots as related to host specificity in the Rhizobium - clover symbiosis, *Applied and Environmental Microbiology* 32, 166-171.

Diatloff, A. (1969). The introduction of Rhizobium japonicum to soil by seed inoculation of non-host legumes and cereals, *Australian Journal of Experimental Agricultural and Animal Husbandry* 9, 357-360.

Elliott, L.F., Gilmour, C.M., Cochran, V.L., Coby, C. and Bennett, D. (1979). Influence of tillage and residues on wheat root microflora and root colonization by nitrogen-fixing bacteria. *In* "The Soil-Root Interface". (Ed. J.L. Harley and R. Scott-Russell), pp. 242-258. Academic Press, London.

Foster, R.C. and Rovira, A.D. (1978). The ultrastructure of the rhizosphere of Trifolium subterraneum L. *In* "Microbial Ecology" (Ed. M.W. Loutit and J.A.R. Miles), pp. 278-290. Springer-Verlag, Berlin.

Fries, N. (1966). Chemical factors in the germination of spores of Basidiomycetes. *In* "The Fungus Spore" (Ed. M.F. Madelin), pp. 189-199. Butterworth, London.

Gibson, A.H. (1966). The carbohydrate requirements for symbiotic nitrogen fixation: A "whole plant" growth analysis approach, *Australian Journal of Biological Science* 19, 499-515.

Griffin, G.J., Hale, M.G. and Shay, F.J. (1976). Nature and quantity of sloughed organic matter produced by roots of axenic peanut plants, *Soil Biology and Biochemistry* 8, 29-32.

Habte, M. and Alexander, M. (1978). Mechanisms of persistence of low numbers of bacteria preyed upon by protozoa, *Soil Biology and Biochemistry* 10, 1-6.

Harley, J.L. (1975). Problems of mycotrophy. *In* "Endomycorrhizas" (Ed. F.E. Sanders, B. Mosse and P.B. Tinker), pp. 1-24. Academic Press, London.

Highinbotham, N. (1968). Cell electropotential and ion transport in higher plants. *In* "Transport and Distribution of Matter in Cells of Higher Plants" (Ed. K. Mothes, I. Muller, A. Nelles and D. Neumann), pp. 167-177. Akademie-Verlag, Berlin.

Iqbal, S.H. and Qureshi, K.S. (1976). The influence of mixed sowing (cereals and crucifers) and crop rotation on the development of mycorrhiza and subsequent growth of crops under field ocnditions, *Biologia* 22, 287-298.

Krupa, S. and Fries, N. (1971). Studies on the ectomycorrhizae of pine 1. Production of volatile organic compounds, *Canadian Journal of Botany* 49, 1425-1431.

Martin, J.K. (1977a). Factors influencing the loss of organic and carbon from wheat roots, *Soil Biology and Biochemistry* 9, 1-9.

Martin, J.K. (1978). The variation with plant age of root carbon available to soil microflora. *In* "Microbial Ecology" (Ed. M.W. Loutit and J.A.R. Miles), pp. 299-302. Springer-Verlag, Berlin.

Marx, D.H. (1969). The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. II. Production, identification, and biological activity of antibiotics produced by Leucopaxillus cereals, var. piceina, *Phytopathology* 59, 411-417.

Meagher, J.W., Brown, R.H. and Rovira, A.D. (1978). The effects of cereal cyst nematode (Heterodera avenae) and Rhizoctonia solani on the growth and yield of wheat. *Australian Journal of Agricultural Research* 29, 1129-1137.

Mosse, B. (1975). Specificity in VA mycorrhizas. *In* "Endomycorrhizas" (Ed. F.E.T. Sanders, B. Mosse and P.B.H. Tinker), pp. 469-484, Academic Press, London.

Newman, E.I. (1978). Root microorganisms : their significance in the ecosystem, *Biological Review* 53, 511-554.

Newman, E.I. and Bowen, H.J. (1974). Patterns of distribution of bacteria on root surfaces, *Soil Biology and Biochemistry* 6, 205-209.

Newman, E.I. and Watson, A. (1977). Microbial abundance in the rhizosphere. A computer model, *Plant and Soil* 48, 17-56.

Ratnayake, M., Leonard, R.T. and Menge, J.A. (1978). Root exudation in relation to the supply of phosphorus and its possible relevance to mycorrhizal formation, *New Phytologist* 81, 543-552.

Reid, C.P.P. and Mexal, J.G. (1977). Water stress effects on root exudation by lodgepole pine, *Soil Biology and Biochemistry* 9, 417-421.

Robinson, R.K. (1972). The production by roots of Calluna vulgaris of a factor inhibitory to growth of some mycorrhizal fungi, *Journal of Ecology* 60, 219-224.

Rovira, A.D., Bowen, G.D. and Foster, R.C. (1980) The nature of the rhizosphere and the influence of the rhizosphere microflora and mycorrhizas on plant nutrition. *In* "Encyclopedia of Plant Physiology, New Series" (Ed. A. Lauchi and R.L. Bieleski), Vol. 12, In Press. Springer-Verlag, Berlin.

Rovira, A.D., Foster, R.C. and Martin, J.K. (1979). Note on terminology: origin, nature and nomenclature of the organic materials in the rhizosphere. *In* "The Soil-Root Interface" (Ed. J.C. Harley and R.S. Russell), pp. 1-4. Academic Press, London.

Rovira, A.D., Newman, E.I., Bowen, H.J. and Campbell, R. (1974). Quantitative assessment of the rhizoplane microflora by direct microscopy, *Soil Biology and Biochemistry*

6, 211-216.

Sanders, F.E., Tinker, P.B., Black, R.L.B. and Palmerley, S.M. (1977). The development of endomycorrhizal roots systems 1. Spread of infection and growth-promoting effects with four species of vesicular-arbuscular endophyte, *New Phytologist* 78, 257-268.

Sivasithamparam, K. and Parker, C.A. (1979). Rhizosphere microorganisms of seminal and nodal roots of wheat grown in pots, *Soil Biology and Biochemistry* 11, 155-160.

Slankis, V. (1973). Hormonal relationships in mycorrhizal development. *In* "Ectomycorrhizae" (Ed. G.C. Marks and T.T. Kozlowski), pp. 231-298. Academic Press, London.

Slope, D.B., Sah, G.A., Broom, E.W. and Gutteridge, R.J. (1978). Occurrence of *Phialophora radicicola* var. *graminicola* and *Gaeumannomyces graminis* var. *tritici* on roots of wheat in field crops, *Annals of Applied Biology* 88, 239-246.

Southwood, T.R.E. (1976). Bionic strategies and population parameters. *In* "Theoretical Ecology, Principles and Applications" (Ed. R.M. May), pp. 26-48. Blackwell, Oxford.

Smith, S.E. and Bowen, G.D. (1979). Soil temperature, mycorrhizal infection and nodulation of Medicago truncatula and Trifolium subterraneum, *Soil Biology and Biochemistry*, In Press.

Sutton, J.C. and Sheppard, B.R. (1976). Aggregation of sand dune soil by endomycorrhizal fungi, *Canadian Journal of Botany* 54, 326-333.

Theodorou, C. and Bowen, G.D. (1971). Influence of temperature on the mycorrhizal associations of Pinus radiata, *Australian Journal of Botany* 19, 13-20.

Theodorou, C. and Bowen, G.D. (1973). Innoculation of seeds and soil with basidiospores of mycorrhizal fungi, *Soil Biology and Biochemistry* 5, 765-771.

Williamson, M. (1972). The analysis of biological populations. Arnold, London.

Wong, P.T.W. and Griffin, D.M. (1976). Bacterial movement at high matric potentials. II. In fungal colonies, *Soil Biology and Biochemistry* 8, 219-223.